THE OSMOTIC PRESSURE OF SEA WATER AND OF THE BLOOD OF MARINE ANIMALS.

Including Some Observations on the Permeability of Animal Membranes.[1]

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Experimental work on the relation of salts and other substances in solution to the life processes of marine animals requires an accurate knowledge of the osmotic pressure of both the sea water and the body fluids of the animals. This knowledge should be supplemented by definite information relative to the permeability of the membranes of the animals under investigation. Thus far this field has been neglected by American investigators. It has been assumed that local conditions are similar to those existing along the European sea board. Owing to the character of the work in our Marine Laboratories it seemed advisable to make some observations which would place our knowledge of local conditions on a firmer basis.

Method.

Until recently our knowledge of sea water and animal fluids has been based solely on quantitative chemical analyses. F. Bottazzi[1] at Naples in 1897 calculated the osmotic pressure from the depression of the freezing point. This method has since been used by several investigators working in the same field (Rodier, Quinton, Frédéricq) and was adopted as most convenient for our purpose. Employing the Beckmann apparatus the freezing point is determined. For aqueous solutions a freezing point below that of distilled water signifies the presence of some substance in solution which is exerting an osmotic pressure. This pressure calculated for 0° C. is equal to about twelve

[1] These investigations were made at the Marine Biological Laboratory, Woods Holl, Mass. They were reported to the Biological Seminar, August 12, 1904.[10] Investigations conducted on the Pacific Coast will be reported later as they are still in progress.

atmospheres for a depression of one degree centigrade (— 1° C.). The depression of the freezing point is designated Δ. Owing to the super-cooling which takes place in solutions as strong as those with which we are dealing, inconstant results are obtained unless freezing is induced by innoculation with a tiny crystal of ice as soon as supercooling of about three tenths of a degree has taken place. A few earlier experiments in which this technique was neglected have not been recorded here. Invertebrate blood clots slowly and the first clot is easily broken up so that the freezing point of fluid as a whole may be determined. Teleost blood was whipped before freezing but no attempt was made to remove the corpuscles in as much as it has been found that they exert no appreciable effect upon the freezing point (Hamburger[11], Hedin[12]).

Osmotic Pressure of Sea Water.

The animals worked with at Woods Hole were obtained from so many different localities that it seemed advisable to determine the freezing point of the water from the same sources. The results of these determinations are given in Table I.

Table I.

	Buzzard's Bay.	Basin of the U. S. Fish Com.	Laboratory Tap.	"Eel Pond."
Maximum Δ	−1.835°	−1.84°	−1.84°	−1.82°
Minimum Δ	−1.81	−1.805	−1.82	−1.75
Average Δ	−1.818	−1.82	−1.82	−1.76
	Eleven samples.	Twenty-three samples.	Forty samples.	Eight samples.

The slight variations noted in the concentration of the different samples of sea water are not due to errors in observation for each sample of sea water was tested repeatedly and the results were checked by the use of three thermometers. The variations may be explained by the more or less land-locked condition of the bodies of water, the concentration being continually, though slightly, altered by the tides and by the continued advent of fresh water. After one extremely heavy rain the water of the laboratory tap showed considerable dilution Δ being — 1.78°. "Eel Pond" water also was diluted till the freezing point was only — 1.70°.

A glance at Table I. shows that the freezing point of the sea water at Woods Hole is on an average of 1.82° C. below zero. The osmotic pressure at a temperature of 0° C. is therefore about 22 atmospheres.

It is a noteworthy fact that the water at Woods Hole is more dilute than is that at Naples (Bottazzi [1]) where $\Delta = -2.29°$. At Arcachon also the sea water is more concentrated, Rodier [21] having determined its Δ to be $-1.89°$ C. According to our determinations made at Pacific Grove, California, the sea water of the Pacific Ocean freezes at $-1.90°$ C.

THE OSMOTIC PRESSURE OF THE BLOOD OF MARINE ANIMALS.

Invertebrates. — The body fluids (or blood) of a number of invertebrates were tested and in every case the freezing point was found to be the same as that of the sea water from which the animal was taken, no variation of more than two hundredths (0.02° C.) of a degree being found. The following list includes the forms worked with and indicates the fluid tested.

Echinodermata :

1. *Thyone briareus* — fluid from the perivisceral cavity.
2. *Arabacia punctulata* — fluid from the perivisceral cavity.
3. *Asterias vulgaris* — fluid from the perivisceral cavity.

Mollusca :

4. *Sycotypus canaliculatus* — blood obtained by section of the foot.
5. *Venus mercenaria* — blood obtained by section of the foot.
6. *Mya arenaria* — blood obtained by section of the foot.

Arthropoda :

7. *Homarus Americanus* — blood.
8. *Limulus polyphemus* — blood.

Selachians. — Two forms were worked with, the blood being obtained from the caudal artery.

TABLE II.

	Maximum Δ.	Minimum Δ.
1. *Mustelus canis*	— 1.90°	— 1.86°
2. Sand shark	2.03	— 1.88

The osmotic pressure of the blood and body fluids of *invertebrates* is due exclusively to the salts which are in solution, the proteid molecules being so large that they exert no appreciable osmotic pressure. Analyses made by L. Frédéricq[7] show that the salts in the blood of a large number of invertebrates are present in the same concentration as in the sea water. Although the blood of *selachians* has a freezing point approximately the same as that of the sea water, the salts are present in much smaller amount, 1.6 per cent. to 2.3 per cent. according to different analyses. The high osmotic pressure is maintained by the presence of a large and variable amount of urea (2–3 per cent., V. Schroeder,[24] Quinton,[20] Rodier,[22] Frédéricq[9]).

Teleosts.—The blood of all teleosts examined showed a low osmotic pressure which, in round numbers, approximated one half that of the sea water.* In Table III. are given the extreme variations in the freezing point for individuals of each species.

TABLE III.

	Source of Blood.	Maximum Δ.	Minimum Δ.
1. Sword fish	Heart after death.	—0.96°	—0.90°
2. Tautog. (*Tautoga onitis*)	Branchial artery.	—0.86	—0.86
3. Squeteague (*Cynoscion regalis*)	" "	—0.864	—0.86
4. Conger eel	" "	—0.82	—0.80
5. Common eel (*Anguilla chrysypa*)	" "	—0.90	—0.90

The results of all these investigations on marine animals agree with those of F. Bottazzi[1] and of Frédéricq[9] at Naples. These investigators found that invertebrate blood froze at —2.03° as did also that of selachians, while teleost blood showed $\Delta =$ —1.04°. The slightly greater depression of the freezing point found by these authors is to be accounted for by a greater concentration of the sea water at Naples than at Woods Hole.

VARIATIONS IN OSMOTIC PRESSURE OF THE BLOOD DUE TO CHANGES IN THE CONCENTRATION ON THE EXTERNAL MEDIUM.

The analyses of L. Frédéricq[7] showed that the concentration of the salts in the blood of invertebrates varied with the concentration

* Incidentally it was observed that the red corpuscles of teleosts were crenated by sea water.

of the salt water from which the animals were taken. This was most strikingly shown by the blood of *Carcinus mœnus* taken from brackish water and from sea water. In the course of our investigations it was found that differences in the freezing point of the blood of invertebrates accompanied differences in the freezing point of the sea water from which they were taken; thus the blood of lobsters taken from the "basin" traps showed $\Delta = -1.82°$, but when taken from the "eel pond" $\Delta = -1.77°$. A decrease in the concentration of the water from the laboratory tap, due to severe rains, caused exactly the same changes in the freezing point of lobster's blood, Δ became $-1.78°$. An increase in the osmotic pressure of the blood of *Limulus* was induced by a two days' exposure to the drying influence of the atmosphere. The freezing point went down to $-1.90°$. The blood of a *Limulus* kept alive for two weeks in a damp cellar froze at $-2.03°$, the normal $\Delta = -1.82°$. Both diurnal and seasonal changes occur in the concentration of the water of San Francisco Bay, Cal. (taken near the Golden Gate), and the perivisceral fluid of starfish shows exactly the same changes; thus on March 17, 1904, $\Delta = -1.47°$ at high tide but at low tide only $-1.385°$. On September 23, $\Delta = -1.80°$.

With these facts as a starting point it was decided to test the freezing point of the blood when the animal was subjected to a large decrease or increase in the osmotic pressure of the external medium.

Dilution of the sea water was first tried and after a longer or a shorter immersion the animal was removed and the freezing point of the blood was determined. The changes which are thus induced are given in Table IV.

In nearly every experiment the animals were kept in the dilute medium until collapse set in, but in a majority of cases they were able to revive when replaced in normal sea water. *Limulus* and *Sycotypus* are particularly hardy and it is noted that the freezing point of their blood changes very quickly until in some cases it is approximately equal to that of the external medium. When the external medium is very dilute death may occur before this equalization takes place; this was particularly true in the case of *Homarus*, which is very susceptible to a change in the con-

TABLE IV.

	Sea Water Diluted with an Equal Volume of Distilled Water, $\Delta = 1.02°$.		Fresh Water, $\Delta = -0.02°$.	
	Duration of Immersion.	Blood Δ.	Duration of Immersion.	Blood Δ.
1. *Asterias*	7 hours.	— 1.14°	12 hours.	much swollen.
2. *Sycotypus*	30 hours.	— 1.07	12 hours.	— 1.23°
			48 hours.	— 0.67
3. *Nereis*	6 hours.	swollen.	3 hours.	swollen.
4. *Chætopterus*	6 hours.	swollen.	3 hours.	swollen.
5. *Limulus*	2.5 hours.	— 1.43	8 hours.	— 1.33
	52 hours.	— 1.12	16 hours.	— .90
6. *Homarus*	2.5 hours.	— 1.43	1 hour.	— 1.63
	6 hrs. (dead).	— 1.32		

centration of the external medium. In these experiments with dilute solutions it was found that when *Venus* was placed in fresh water it closed up so tightly that after two weeks' immersion the osmotic pressure of the blood was not lowered; perforation of the edges of the shell however admitted the fresh water and the osmotic pressure was lowered. A lobster placed in 3 vol. sea water + 1 vol. distilled water showed a lowering of the osmotic pressure, as indicated by $\Delta = -1.46°$. Marine flat worms on the bodies of Limuli became much swollen in the diluted sea water although they remain active for a long time.

Concentrated sea water obtained by evaporating until $\Delta = -3.80°$ was next used as the medium of immersion. The blood of *Limulus* after sixteen hours in this medium froze at $-3.79°$. The blood of *Homarus* in this doubly concentrated sea water froze at $-3.60°$ at the end of eight hours. In sea water concentrated to 0.8 its original volume the blood of *Homarus* froze at $-2.17°$ after two hours' immersion. These animals tolerate an increased osmotic pressure much better than the equivalent decrease. When the aquarium water is concentrated, complete equalization of enormous differences in the osmotic pressure between the external and internal media may take place without any marked symptoms of asthenia such as are seen when the external medium is dilute.

From the facts just sketched we see that all of the marine invertebrates which we have worked with are truly "poikilosmotic." Two factors may be at work in producing the variations

in the osmotic pressure, viz., the interchange of water, and of salts.

The entrance of water is proven by the enormous increase in weight, and the swollen appearance of those animals which have been placed in diluted sea water. This swelling has already been referred to (Table IV.) as noticeable in *Asterias* and the two worms *Nereis* and *Chætopterus*. In one experiment in which a *Limulus* was kept for sixteen hours in fresh water the animal became so swollen that the gills burst and the water of the aquarium became blue from the hæmocyanin of the exuded blood. The blood of the animals subjected to diluted media became noticeably less viscous and owing to its increased volume and the high internal pressure, was easily obtained from the animal. When subjected to concentrated sea water it was often difficult to secure sufficient blood from the lobster to make the desired determinations. That an exchange of salts also takes place, although far less rapidly than the exchange of water, is shown by the fact that when the animals are placed in distilled water, chlorides are eliminated and a copious precipitate is obtained upon the addition of silver nitrate. No quantitative chemical analyses of the aquarium water were made but in one such case an increase in the osmotic pressure was indicated by the change in the freezing point; *Limulus* was the animal experimented with and after twelve hours' immersion the freezing point of the aquarium water had been lowered from $-0.02°$ to $-0.23°$.

Quinton,[18, 19] experimenting with *Aplysia*, has also found an increase in weight when the animal is subjected to dilute sea water and a loss in weight in concentrated solutions, and he has further, by chemical analyses, found loss and gains in the amount of salts in the blood of this animal just equalling the respective gain or loss from the aquarium water. Frédéricq[3, 7, 8] made similar analyses.

There are many other proofs of the permeability of the invertebrate membranes to various salts. Loeb's[15] experiments on the rhythmic contractions of medusæ (*Gonionemus*) indicate the permeability to NaCl, $CaCl_2$, and KCl. The death of invertebrates is easily induced by acids and the salts of the heavy metals. Frédéricq[9] has placed potassium ferrocyanide and nitrates in the

aquarium water and later obtained positive tests for these substances in the blood of *Carcinus mœnus*. We may conclude then that the membranes of marine invertebrates as a class are permeable both to water and to salts and act exactly "like a dialyzer membrane."

The path taken by the exchanged material is not so certainly known. Frédéricq[6] assumes that the branchial membranes of *Carcinus mœnus* are the permeable structures but publishes no evidence supporting the view. Quinton[19] takes it for granted that it is the external wall which is permeable, and this seems to be true for *Aplysia*, the form with which he worked, as has been shown by Ph. Bottazzi and P. Enriquez.[2]

That the outer wall is the permeable structure of worms may be shown by a very simple experiment performed by the author on *Nereis* and *Chætopterus*. Ligatures were passed about the animals close to either end and drawn tight thus completely closing the alimentary canal; care was taken to avoid abrasion of the integument. When placed either in fresh water or dilute sea water swelling and increase in weight was obtained.

Limulus is an animal on which the permeability of the gills may be easily demonstrated. These structures are borne on the abdominal segment which may be bent ventrally to an angle of about 90°. When placed in this position and so propped up in the aquarium that only the abdomen is under the surface of the water, the mouth parts may be as much as fifteen centimeters above the surface. No water can enter the alimentary canal, nevertheless in equal parts of fresh water and sea water ($\Delta = 1.03°$), six hours sufficed to render the integument and gills tense and swollen. A freezing-point determination showed that Δ of blood had changed from $-1.82°$ to $-1.32°$. In another experiment with *Limulus* the animal was placed astride a narrow dish of fresh water and so supported that only the gills dipped beneath the surface with each rhythmic oscillation. After eight hours enough water had been absorbed to bring Δ down to $-1.41°$, the freezing point of the water had also changed from $-0.02°$ to $-0.20°$, and a copious precipitate of silver chloride was obtained. The gills of *Limulus* are permeable both to water and to salts. Metals in proteid combination, *e. g.*, copper of hæmo-

cyanin, of course do not diffuse owing to the enormous size of the molecules with which they are incorporated.

Selachians (*Mustelus canis*). — Dog fish kept in fresh water for one hour showed signs of asphyxia and were removed in a dying condition. The defibrinated blood showed a considerable decrease in osmotic pressure, Δ being changed to $-1.45°$ C. After three hours' immersion in sea water diluted with an equal volume of distilled water the blood froze at $-1.60°$ C.

These experiments demonstrate the permeability of the membranes to water. As has already been pointed out, the composition of the selachian blood is very different from sea water in its salt content, but the osmotic pressure is maintained by the presence of large quantities of urea in the blood. It is evident then that selachian membranes are semi-permeable. Little more than this can be said, for this group has not been sufficiently investigated. The same may be said of the cyclostomes. Since these animals are found in both fresh and salt waters, and some species migrate at certain seasons from salt into fresh water, the author is making a more careful study of these groups. Mosso [17] found that selachian red-blood corpuscles were laked in a 2.5 per cent. solution of sodium chloride, and that when a selachian (*Scyllium*) was placed in fresh water death resulted in a few hours. He describes a disintegration of the blood corpuscles with the formation from their débris of a sort of coagulum which plugs up the branchial arteries with consequent death from suffocation. Death resulted in one half hour if the tails had been cut off before immersion in the fresh water.

Teleosts. — In nature we have experiments of the sort under consideration, in the movements of such fish as the eels and salmon, which go from salt into fresh water at the spawning season. We have as yet no data relative to changes in osmic pressure of the blood coincident with these migrations. The author experimented with *Anguilla chrysypa*. This form lives equally well in salt water or fresh-water aquaria and tolerate sudden transmission from one medium to the other without apparent injury. In testing the freezing point of the blood in different media variations were found, but it was impossible to attribute them to the actual changes in osmotic pressure, for similar variations were found in

different tests made with animals taken from the same medium. In all these experiments it was necessary to sacrifice several animals to get even a minimum amount of blood for making determinations, so that the results were on the whole unsatisfactory; still, they suffice for the conclusion that only a slight, if any, change is induced by a change in the osmotic pressure of the external medium. The animals are "homoiosmotic."

Fundulus heteroclitus is a hardy little fish well suited to this form of experimentation, although the quantity of the blood is too small to admit of making cryoscopic determinations. It was found that if care was taken to select individuals which were not injured in catching, about eighty per cent. lived in fresh water for six weeks when the experiment was discontinued. This is as high a percentage as can be kept alive in the sea water aquaria of the laboratory. When placed in external media of concentrations varying from fresh water to sea water concentrated to twice its normal strength they showed the same hardihood. It is reasonably certain that the osmotic pressure of the blood does not change to any marked degree for an examination of the blood corpuscles did not show either laking in the dilute media or crenation in the media of higher concentration. The integument and gills are therefore impermeable. Loeb [14, 16] has found that *Fundulus* embryos will live in distilled water and in sea water to which 5 per cent. NaCl has been added.

The view that the membranes of these fish are completely permeable (Brown) [25] is not tenable, at least concerning adult *Fundulus*, as is shown by the following series of experiments which were repeated often enough to assure the verity of the results.

A large number of healthy specimens were selected and about one half the body surface denuded of scales by gentle scraping with the edge of a scalpel, or the skin was removed over an area of one square centimeter on each side; then they were divided into three lots and placed respectively into fresh water, sea water diluted with an equal volume of distilled water, and normal sea water. Of those kept in fresh water in every experiment from eighty to ninety per cent. died within twenty-four hours while all died in less than thirty-six hours. In normal sea water the fish suffered a similar fate although death did not intervene so soon.

But of those kept in sea water of one half its normal concentration only three per cent. were dead at a time when all those in the other two media had died, and seventy per cent. were kept alive for four weeks, when the wounds were all healed and the experiments discontinued. In these experiments therefore, no deleterious effects obtain when the internal and external media are approximately isotonic—in spite of the injuries and free interchange between blood and aquarium water. In the hypotonic and hypertonic solutions, however, distinct changes resulting in death, take place. In the strong solutions (normal sea water) microscopic examination showed that the blood corpuscles were crenated. In fresh water the fish became greatly swollen indicating the absorption of water. Whether laking or swelling of the corpuscles takes place was not determined in this series of experiments.*

From these experiments we may conclude that in all probability the blood of *Fundulus* does not suffer much if any change in concentration when the fish is transferred from salt water into fresh water or vice versa, provided the membranes are uninjured. If these experiments admit of general application to migratory teleosts they would indicate that these animals also are in some way protected from changes in the osmotic pressure of the blood and tissues and that the principal protective factor probably lies in a lack of permeability of their membranes. We may further conclude that in case of serious abrasion to the integument the membranes become permeable and a change of osmotic pressure of the blood results, a change which may induce the death of the animal. The great mortality of the salmon after spawning in the head waters of California's streams, is a well-known fact (Rutter [23]). Whether the generally battered condition of these fish at the spawning season bears any relation to changes in the osmotic pressure of the blood has not been investigated. It is not impossible that the actual cause of death lies in a decrease in the osmotic pressure of the blood and that the injuries are responsible for death only in so far as they permit the entrance of water and de-

*The hæmatocrit would doubtless prove a valuable aid in making experiments of this sort when it is impossible to obtain sufficient blood for freezing point determinations

crease the osmotic pressure of the blood. In consequence of such an event we would expect nutritional changes and possibly a disintegration of the red blood corpuscles. Such a disintegration in many marine teleosts has been described though the resistance of blood corpuscles of the migratory fish seems to be very much greater than of other forms of fish, for they lose their hæmoglobin only in salt solutions containing as low as 0.3–0.4 per cent. sodium chloride (Mosso [17]).

The maintenance of an osmotic pressure lower than that of the sea water speaks for a relative impermeability of all the membranes of marine teleosts. The experiments we have described indicate an absolute impermeability of the integument. It may not be assumed that all the membranes are absolutely impermeable nor is there the same degree of impermeability in all teleosts as is indicated by the great variation in the resistance of marine teleosts to changes in the osmotic pressure of the aquarium water. Loeb's [14] experiments with *Fundulus* on the poisonous effects of sodium chloride indicate a certain degree of permeability of some surface. The poisonous action of heavy metallic salts, of acids, and alkalis indicate the same fact although many other factors must be taken into consideration among which is a possible and probable alteration of permeability due to these chemicals. A certain degree of permeability of young *Fundulus* is prettily shown by immersing them in sea water containing the merest trace of colored salts, such as salts of copper, cobalt and manganese. In these cases the solution may be almost colorless but in time the living embryos may become very deeply colored.

The facts indicate the probability of some regulative mechanism among teleosts (Höber [13]) the existence of which has not yet been demonstrated, and the nature of which cannot be affirmed.

Summary.

1. The sea water at Woods Hole, Mass., freezes at — 1.82° C., at Pacific Grove, Cal., Δ is — 1.90° C.

2. The blood or body fluid of a marine invertebrate has the same freezing point as the sea water from which it is taken, and therefore has the same osmotic pressure. This is also true of selachian blood, although the salt content is lower than that of

the sea water, the deficit of salts in the blood of this latter group is compensated by the osmotic pressure of the urea in the blood.

3. The osmotic pressure of teleost blood is about half that of the sea water ($\Delta = -0.8°$ to $-0.96°$ C.).

4. A dilution or concentration of the aquarium water always causes an equivalent change in the blood of invertebrates, and osmotic equilibrium between "internal and external media" is established. Their membranes are completely permeable. This permeability is proven for the integument of worms and the gills of *Limulus*.

5. Dilution or concentration of the aquarium water causes a change in the same sense in the blood of selachians, but death ensues before osmotic equilibrium is established. The membranes of selachians are semi-permeable.

6. Only slight if any change takes place when teleosts (*Anguilla*) are transferred from salt to fresh water and *vice versa*. Normal *Fundulus heteroclitus* will live in water varying in osmotic pressure from that of the tap to sea water concentrated to double its strength. The membranes of teleosts are impermeable, or the fish possess some regulative mechanism which keeps the osmotic pressure of the blood nearly constant. Extensive abrasion of the skin of *Fundulus* results in death in aquarium water of less or greater osmotic pressure than that of their blood, for example they die in fresh water and in *normal* sea water but not in sea water diluted with an equal volume of distilled water.

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